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# ABA-mediated transcriptional regulation in response to osmotic stress in plants

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**Abstract** The plant hormone abscisic acid (ABA) plays a pivotal role in a variety of developmental processes and adaptive stress responses to environmental stimuli in plants. Cellular dehydration during the seed maturation and vegetative growth stages induces an increase in endogenous ABA levels, which control many dehydration-responsive genes. In Arabidopsis plants, ABA regulates nearly 10% of the protein-coding genes, a much higher percentage than other plant hormones. Expression of the genes is mainly regulated by two different families of bZIP transcription factors (TFs), ABI5 in the seeds and AREB/ABFs in the vegetative stage, in an ABA-responsive-element (ABRE) dependent manner. The SnRK2-AREB/ABF pathway governs the majority of ABA-mediated ABRE-dependent gene expression in response to osmotic stress during the vegetative stage. In addition to osmotic stress, the circadian clock and light conditions also appear to participate in the regulation of ABA-mediated gene expression, likely conferring versatile tolerance and repressing growth under stress conditions. Moreover, various other TFs belonging to several classes, including AP2/ERF, MYB, NAC, and HD-ZF, have been reported to engage in ABA-mediated gene expression. This review mainly focuses on the transcriptional regulation of ABA-mediated gene expression in response to osmotic stress during the vegetative growth stage in Arabidopsis.

**Keywords** Abscisic acid (ABA) · Arabidopsis · Dehydration · Osmotic stress · Stress response · Transcription factor

## Introduction

The plant hormone abscisic acid (ABA) plays a key role in a broad array of developmental processes and adaptive stress responses to environmental stimuli in plants (for reviews: Cutler et al. 2010; Finkelstein et al. 2002; Hubbard et al. 2010; Nambara and Marion-Poll 2005; Raghavendra et al. 2010; Wasilewska et al. 2008; Weiner et al. 2010). In particular, ABA appears to be closely associated with cellular dehydration processes in the seed maturation and vegetative growth stages. Numerous dehydration-responsive genes have been reported to date, and many can be induced by exogenous ABA application (Yamaguchi-Shinozaki and Shinozaki 2006; Zhu 2002). The application of exogenous ABA has often been used to mimic dehydration responses (Bartels and Sour 2004), although it is generally accepted that both ABA-dependent and ABA-independent pathways are involved in dehydration responses (Shinozaki et al. 2003; Yamaguchi-Shinozaki and Shinozaki 2005). ABA-responsive gene expression is directly regulated by transcription factors (TFs) that

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recognize and bind to *cis*-elements in the promoter regions upstream of their target genes. In addition to these TFs, ABA-mediated gene expression is controlled by receptors, secondary messengers, protein kinase/phosphatase cascades, and chromatin-remodeling factors. Moreover, miRNA targeting, mRNA maturation and stability, and protein degradation all appear to modulate ABA-responsive gene expression. Recently, there has been an exponential increase in the number of studies concerning ABA-mediated gene expression in plants. This review mainly focuses on the transcriptional regulation of ABA-mediated gene expression in response to osmotic stress during the vegetative growth stage of *Arabidopsis thaliana*, which is a representative model plant.

## ABA-mediated gene expression appears to confer versatile tolerance and to repress growth under osmotic stress conditions in a circadian-dependent manner

Increased ABA levels in response to osmotic stresses, such as drought and high salinity, induce the expression of many genes that appear to play multifaceted roles in dehydration response and tolerance in both vegetative tissues and seeds (Finkelstein et al. 2002; Yamaguchi-Shinozaki and Shinozaki 2006). Expanding transcriptome data sets have uncovered a global picture of ABA-regulated genes in Arabidopsis plants (Cutler et al. 2010; Fujita et al. 2009b; Goda et al. 2008; Kilian et al. 2007; Nemhauser et al. 2006; Seki et al. 2002; Shinozaki et al. 2003; Urano et al. 2009). ABA-induced genes include those that encode proteins associated with stress response and tolerance, including late embryogenesis abundant (LEA) proteins; a number of regulatory proteins, such as TFs, protein kinases, and phosphatases; a variety of transporters; and enzymes involved in osmoprotectant synthesis, phospholipid signaling, fatty acid metabolism, cellular metabolism, carbohydrate metabolism, and secondary metabolism. Conversely, the genes down-regulated by ABA are enriched for those encoding proteins involved in growth and development, such as ribosomal, chloroplast, cell wall, and plasma membrane proteins. Moreover, the integration of transcriptome and metabolome data sets has revealed that drought-induced ABA-dependent transcriptional regulation plays a pivotal role in carbohydrate metabolism (Kempa et al. 2008) and in the biosynthesis of branched-chain amino acids, saccharopine, proline and polyamine (Urano et al. 2009).

Nearly 10% of the protein coding-genes in Arabidopsis are likely to be regulated by ABA (Nemhauser et al. 2006). It has been shown that ABA regulates a much larger subset of genes than other plant hormones (Goda et al. 2008; Mizuno and Yamashino 2008; Nemhauser et al. 2006). These findings suggest that ABA-mediated gene expression plays a versatile and pivotal role in plants. Interestingly, the expression of many ABA-responsive genes appears to oscillate diurnally in a robust manner (Mizuno and Yamashino 2008). Consistent with this finding, ABA has been reported to accumulate in a diurnal pattern (Nováková et al. 2005) and to control circadian period in a light dependent manner (Hanano et al. 2006). These findings are also in accordance with the recent observation that the bZIP TF HY5, which is a positive mediator of light-regulated genes, integrates the ABA and light signaling pathways, partially through direct activation of ABI5 (Chen et al. 2008). Recently, TOC1 has been identified as a molecular switch connecting the circadian clock to ABA-mediated transcription and drought stress responses (Legnaioli et al. 2009). In addition, 68% of the circadian-regulated genes are reported to be stress-responsive (Kreps et al. 2002). Collectively, these observations may support the view that the circadian clock mediates a plant's ability to adapt to daily changes in water status by controlling endogenous ABA levels and subsequent ABA-responsive gene expression.

## ABRE is the major *cis*-element in ABA-induced gene expression

Most ABA-regulated genes contain conserved ABA-responsive elements (ABREs; PyACGTGG/TC) as the determinant *cis*-elements in their promoters (Busk and Pagès 1998; Gómez-Porras et al. 2007; Hattori et al. 2002; Zhang et al. 2005). These elements belong to the G-box (CACGTG) family, which has been implicated in a wide range of gene expression mechanisms in plants (Menkens et al. 1995). ABREs contain an ACGT core, which has shown to be recognized by plant bZIP proteins (Choi et al. 2000; Foster et al. 1994; Hattori et al. 1995; Hobo et al. 1999; Uno et al. 2000). In general, a single copy of ABRE is not sufficient to confer ABA-mediated induction of transcription. Successful ABA-induced gene expression requires either additional copies of the ABRE or coupling elements (CEs; Hobo et al. 1999; Shen et al. 1996; Skriver et al. 1991). To date, *CE1*, *CE3*, *motifIII*, and *DRE/CRT* have been identified as CEs (Busk and Pagès 1998; Narusaka et al. 2003; Shen et al. 1996). Recently, however, a frequency distribution approach has shown that ABRE–ABRE pairs are major *cis*-elements in Arabidopsis and rice (Gómez-Porras et al. 2007). Moreover, it appears that these motifs need to be located close to the transcription start site of the genes (Berendzen et al. 2006). The other regulatory elements associated with ABA-induced gene expression will be discussed below in the section corresponding to each TF.

## ABA regulates many non-AGI transcriptional units and induces chromatin remodeling under osmotic stress conditions

Latest approaches using tiling arrays and chromatin immunoprecipitation (ChIP) analyses have provided a new picture of ABA-mediated transcriptional regulation. Recent transcriptome analyses with Arabidopsis whole-genome tiling arrays have revealed that in addition to the ABA-regulated protein-coding genes, ABA regulates 5–10% of the roughly 8,000 non-AGI transcriptional units (TUs) that have been identified in the "intergenic" regions of the Arabidopsis genome (Matsui et al. 2008; Zeller et al. 2009). Most of these TUs are likely to be composed of fully overlapping sense–antisense transcripts in which the expression of the sense TUs is required for the ABA-induced expression of the antisense TUs. Intriguingly, a large number of such TUs lack any known ABREs upstream of their 5′ ends, suggesting that ABREs are not involved in the expression of these TUs.

A recent ChIP technique has also revealed the involvement of chromatin modulation in ABA-mediated transcriptional regulation (Kim et al. 2010). Several kinds of modification on the N-terminal domain of the histone H3 appear to occur at the chromosomal regions of ABA-responsive marker genes, such as *RD29B* and *RD20*, in response to osmotic stress (Kim et al. 2008). This finding is consistent with similar observations from conventional protein-blot analyses (Sokol et al. 2007). Several chromatin-regulation related factors have been reported to be associated with ABA-dependent gene expression in plants, including elongators of the histone acetyltransferase (HAT) complex (ELP1/ABO1/ELO2, ELP2, ELP4/ELO1, and ELP6: Zhou et al. 2009), an ATP-dependent chromatin remodeling factor (SWI3B: Saez et al. 2008), a linker histone H1 (HIS1-3: Ascenzi and Gantt 1997; Fujita et al. 2005), and a histone deacetylase (HD2C: Sridha and Wu 2006). Excellent review articles in this area have been published recently (Chinnusamy et al. 2008; Kim et al. 2010).

## AREB/ABFs are the major TFs controlling ABA-mediated ABRE-dependent gene expression

TFs are thought to play a central role in controlling ABA-responsive gene expression by interacting with *cis*-elements in the promoter regions upstream of the target genes in response to osmotic stress during the vegetative growth stage of Arabidopsis (Fig. 1). Among the large number of such TFs that have been identified to date, the bZIP TFs belonging to the AREB/ABFs family have been shown to be major TFs that control ABA-mediated gene expression under osmotic stress conditions.

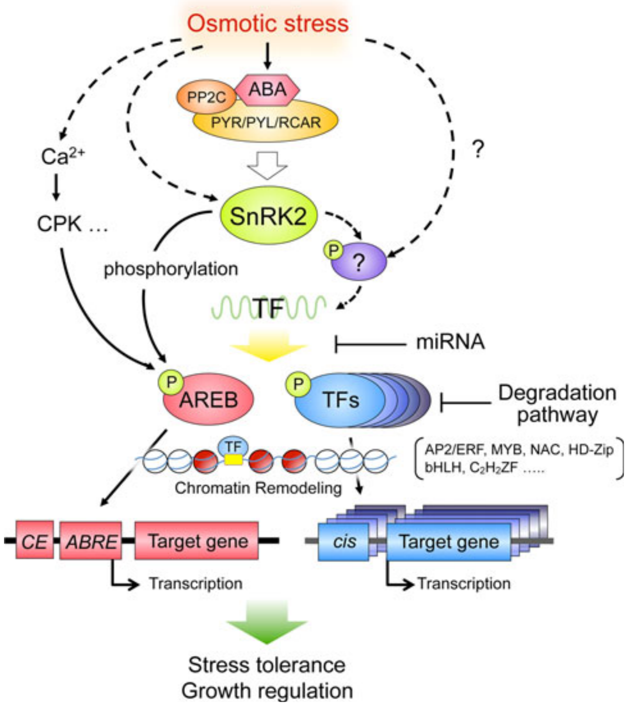

**Fig. 1** A working model of ABA-mediated transcriptional regulation in response to osmotic stress. PYR/PYL/RCAR ABA receptor-PP2C complexes control SnRK2-AREB/ABF pathways to regulate major ABA-mediated ABRE-dependent gene expression. TFs regulate ABA-mediated gene expression by interacting with *cis*-elements in the promoter regions upstream of the target genes in response to water stress during vegetative growth in Arabidopsis. *Broken lines* indicate possible but not firmly demonstrated routes. *TF* transcription factor, *PP2C* protein phosphatase 2C. See main text for details

The TFs of the bZIP family regulate pivotal cellular processes in all eukaryotes (Corrêa et al. 2008; Jakoby et al. 2002). The bZIP domain consists of two motifs: a basic region implicated in the specific binding of the TF to its target DNA and a leucine zipper responsible for TF dimerization. The Arabidopsis genome contains more than 75 members of the bZIP family, which have been classified into more than ten groups based on sequence similarity. Interestingly, the well-studied bZIP TFs involved in ABA signaling are concentrated in Group A, which has 13 members. Two N-terminal conserved domains, C2 and C3, are seen in all 13 members of Group A. However, four members [FD (Abe et al. 2005), FDP (Abe et al. 2005), GBF4 (Menkens and Cashmore 1994), and AtbZIP27] do not contain any recognizable form of the C1 domain, which is well conserved in the other nine members that are considered to be involved in ABA signaling (Bensmihen et al. 2002). Based on phylogenetic relationships (Bensmihen et al. 2002; Yoshida et al. 2010), family members carrying all three N-terminal conserved domains can be divided into two groups. The five *ABI5/AtDPBF* family genes (*ABI5*, *EEL*, *DPBF2/AtbZIP67*, *DPBF4*, and *AREB3*) are mainly expressed in seeds and appear to play important roles in seed maturation and development (Bensmihen et al. 2005; Bensmihen et al. 2002; Finkelstein and Lynch 2000; Kim 2006; Kim et al. 2002; Lopez-Molina and Chua 2000), whereas the *AREB/ABF* family genes (*AREB1/ABF2*, *AREB2/ABF4*, *ABF1*, and *ABF3*) are mainly expressed in vegetative tissues under abiotic stress conditions (Choi et al. 2000; Fujita et al. 2005; Kang et al. 2002; Kim et al. 2002; Uno et al. 2000) (Fig. 2).

These four AREB/ABF proteins were originally identified as ABRE-binding proteins (AREB: Uno et al. 2000) or ABRE-binding factors (ABF: Choi et al. 2000) using the yeast one-hybrid screening method with cDNA libraries prepared from Arabidopsis seedlings. While *ABF1* is significantly induced by cold (Kim 2006) but not by osmotic stress (Fujita et al. 2005), the other three *AREB/ABF* genes (*AREB1/ABF2*, *AREB2/ABF4*, and *ABF3*) are induced both by ABA and by osmotic stresses such as high salinity and dehydration and have been shown to be key regulators of ABA signaling in response to osmotic stress during the vegetative growth stage (Abdeen et al. 2010; Fujita et al. 2005; Kang et al. 2002; Kim et al. 2004b; Uno et al. 2000; Yoshida et al. 2010). Analyses of the over-expression of AREB1/ABF2, AREB2/ABF4, or ABF3 in transgenic plants have shown that all three of these AREB/ABF TFs mediate stress-responsive ABA signaling; AREB1/ABF2 is also likely implicated in glucose signaling (Kang et al. 2002; Kim et al. 2004b). Transient expression assays using protoplasts prepared from wild-type (WT) or ABA-deficient (*aba2*) mutant plants have revealed that AREB1/ABF2, AREB2/ABF4, and ABF3 all require ABA for full activation (Fujita et al. 2005; Uno et al. 2000; Yoshida et al. 2010). In fact, the ectopic expression of AREB1/ABF2 in transgenic Arabidopsis plants is not sufficient to activate the target genes, such as *RD29B* (Fujita et al. 2005). However, the over-expression of an active form of

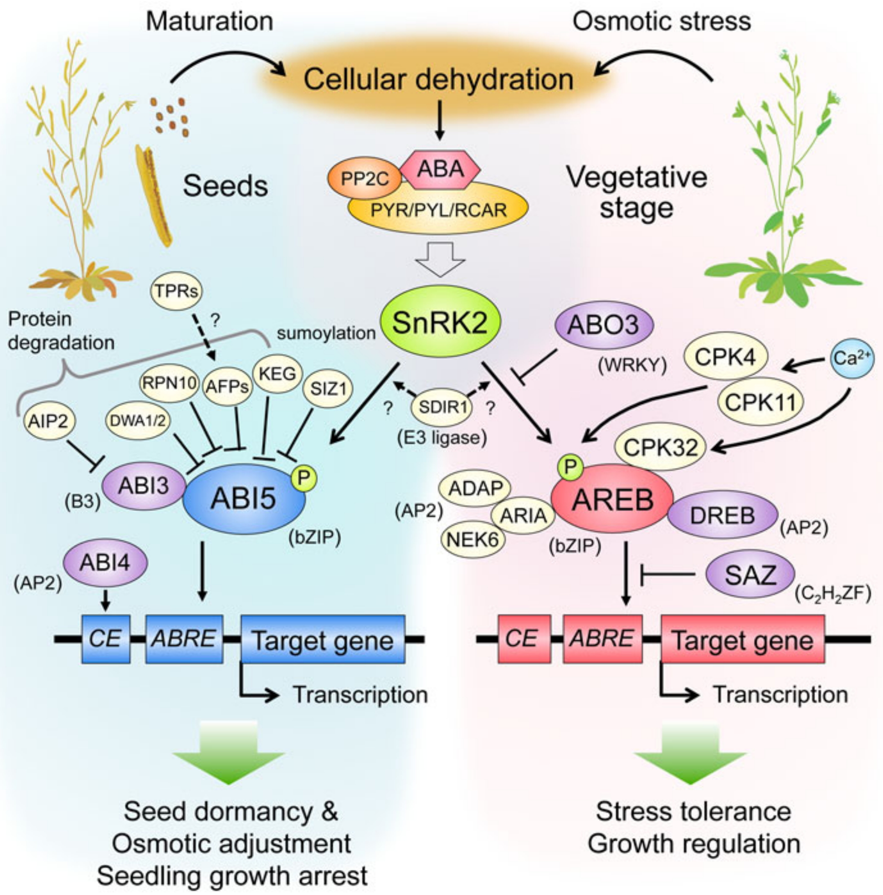

**Fig. 2** A schematic model of the transcriptional regulation of ABA signaling by AREB/ABF and ABI5 family TFs. Two different bZIP family TFs (ABI5 and AREB/ABFs) play key roles in ABA-mediated ABRE-dependent gene expression associated with similar cellular dehydration in seeds and in the vegetative stage through different bZIP modulation mechanisms, in addition to the common core sensing system (PYR/PYL/RCAR-PP2C-SnRK2). *Broken lines* indicate possible but not firmly demonstrated routes. See main text for details

AREB1/ABF2 harboring an internal deletion, but retaining the N-terminal activation and C-terminal bZIP DNA-binding domains, activates target genes, including *RD29B*, and enhances both the sensitivity to ABA and tolerance to drought stress in transgenic Arabidopsis plants (Fujita et al. 2005). In contrast, *AREB1/ABF2*-repressed plants exhibit the opposite phenotype (Fujita et al. 2005). The three AREB/ABF proteins that can form homo- and heterodimers (AREB1/ABF2, AREB2/ABF4, and ABF3) appear to have largely overlapping functions (Yoshida et al. 2010). An *areb1 areb2 abf3* triple mutant displays enhanced ABA insensitivity and reduced tolerance to drought stress in comparison to single and double knockout mutants of the AREB/ABF TFs (Yoshida et al. 2010). Large-scale transcriptome analysis has shown that stress-responsive gene expression is remarkably impaired in the triple mutant and has revealed novel ABRE-dependent AREB/ABF target genes in response to osmotic stress, including many LEA class and group-Ab protein phosphatase 2C (PP2C) genes and TFs (Yoshida et al. 2010). Collectively, these data demonstrate that AREB1/ABF2, AREB2/ABF4, and ABF3 are master transcription factors that cooperatively regulate ABA-mediated ABRE-dependent gene expression under osmotic stress conditions.

## AREB/ABF phosphorylation by SnRK2 protein kinases

In-gel kinase assays have shown that the activation of AREB/ABF proteins requires for the phosphorylation of multiple RXXS/T sites in the conserved regions by SnRK2 protein kinases in an ABA-dependent manner (Furihata et al. 2006; Uno et al. 2000). When the Ser/Thr residues at the putative phosphorylation target sites in the conserved region of the AREB1/ABF2 protein are substituted with Asp to mimic the negative charge of the phosphorylated residues, the resulting dominant active form of AREB1 activates the expression of many downstream ABA-responsive genes, even in the absence of ABA (Furihata et al. 2006). Three SNF1-related protein kinases (SnRK2s; SRK2D/SnRK2.2, SRK2E/SnRK2.6, and SRK2I/SnRK2.3) and AREB/ABFs appear to co-localize and interact in plant cell nuclei (Fujita et al. 2009c; Yoshida et al. 2010). The *srk2d srk2e srk2i* triple mutation has been reported to completely impair the ABA-activated phosphorylation of AREB/ABFs in vitro (Fujii et al. 2009; Fujii and Zhu 2009). Furthermore, the down-regulated genes in the *areb1 areb2 abf3* and *srk2d srk2e srk2i* triple mutants largely overlap in ABA-dependent expression in response to osmotic stress (Fujita et al. 2009c). All of these findings support the notion that the three subclass III SnRK2s control AREB/ABFs in ABA-responsive gene expression under osmotic stress conditions.

The RXXS/T phosphorylation target sites that have been identified in the ABA-dependent phosphorylation of AREB/ABFs by SnRK2s are also known as the putative target sites of general Ser/Thr protein kinases, such as calcium-dependent protein kinases (CPKs) (Furihata et al. 2006; Kagaya et al. 2002). In fact, it has been reported that CPK32 physically interacts with AREB2/ABF4 (Choi et al. 2005). In addition, CPK4 and CPK11, which phosphorylate AREB2/ABF4 and ABF1 in an ABA-dependent manner in vitro, appear to be positive regulators of ABA signaling at the whole-plant level (Zhu et al. 2007). Thus, these CDPKs may partially participate in the phosphorylation of AREB/ABFs, in addition to SnRK2s. Recently it has been shown that the binding of bZIP TFs, HY5 and AtbZIP63, to ACGT boxes depends on the phosphorylation state of conserved residues inside of the DNA-binding domains (Kirchler et al. 2010). Collectively, it appears that phosphorylation of bZIP TFs plays various important roles in the regulation.

## Global regulators of ABA-dependent gene expression: the SnRK2-AREB/ABF pathway governs the major ABA-mediated ABRE-dependent gene expression in response to osmotic stress

A new model for ABA signaling has been proposed and verified. In this model, PYR/PYL/RCAR ABA receptor-PP2C complexes control SnRK2-AREB/ABF pathway to regulate the major ABA-mediated ABRE-dependent gene expression (for reviews: Cutler et al. 2010; Hubbard et al. 2010; Raghavendra et al. 2010; Weiner et al. 2010). In the presence of ABA, PYR/PYL/RCAR family START proteins, which act as ABA receptors, recognize and bind to group-A PP2C molecules. Subclass III SnRK2s (SRK2D/SnRK2.2, SRK2E/SnRK2.6, and SRK2I/SnRK2.3) are then released from PP2C-dependent negative regulation, allowing the SnRK2s to regulate downstream TFs, including AREB/ABFs, activating the expression of many ABA-responsive genes (Fig. 1).

Disruption of the three SnRK2 protein kinases completely blocks almost all ABA responses (Fujii and Zhu 2009; Fujita et al. 2009c; Nakashima et al. 2009). Interestingly, although these SnRK2s are considered to directly target (Fujita et al. 2009c; Yoshida et al. 2010) and phosphorylate AREB/ABFs in vitro (Fujii et al. 2007, 2009; Fujii and Zhu 2009) and in vivo (Kline et al. 2010) to regulate downstream target genes, the triple mutation also remarkably blocks the ABA-induced transcription of many TFs, such as AREB1/ABF2 and ABF3 (Fujita et al. 2009c). In fact, the post-transcriptional modification of AREB/ABFs has been demonstrated, whereas little is known about how the expression of AREB/ABFs and the other ABA-inducible TFs are upregulated. Moreover, the mechanisms underlying the activation of SnRK2 and PP2C-dependent suppression of SnRK2 activity remain unclear. However, taken together with the observation that the SnRK2-AREB/ABF pathways appear to be highly conserved in rice (Kagaya et al. 2002; Kobayashi et al. 2004, 2005), phylogenetic analyses (Corrêa et al. 2008; Yoshida et al. 2010) support the notion that the SnRK2-AREB/ABF system is a major positive regulatory system that is ubiquitous and well conserved in embryophytes.

## Differential roles and modulation of AREB/ABF and ABI5 family TFs in ABRE-dependent transcription associated with cellular dehydration in seeds and in the vegetative stage

It has been shown that AREB/ABF bZIP TFs regulate ABRE-dependent gene expression during osmotic stress conditions, whereas ABI5 family members are considered to control ABRE-mediated transcription mainly in seeds. Seed maturation and osmotic stress during the vegetative growth stage cause cellular dehydration, thereby increasing endogenous ABA levels in plants (Finkelstein et al. 2002; Yamaguchi-Shinozaki and Shinozaki 2006). ABA appears to allow subclass III SnRK2s to phosphorylate and activate ABI5 family TFs to regulate ABRE-dependent gene expression (Finkelstein et al. 2005; Fujii et al. 2007; Fujii and Zhu 2009; Kobayashi et al. 2005; Nakashima et al. 2009). This mechanism appears to enhance the osmotic adjustment and dormancy of seeds, as well as seedling growth arrest (Miura et al. 2009) (Fig. 2). Genetic analyses have shown that ABI5 is genetically epistatic to ABI3 (Finkelstein and Lynch 2000; Lopez-Molina et al. 2002). Several negative regulators of ABI5 have been reported to date. The ABI5-binding proteins, AFPs (Garcia et al. 2008; Lopez-Molina et al. 2003); a RING finger ubiquitin E3 ligase, KEG (Liu and Stone 2010; Stone et al. 2006); and two CUL4-based E3 ligases, DWA1 and DWA2 (Lee et al. 2010a), appear to be involved in ABI5 proteasome degradation. In addition, a 26S proteasome subunit, RPN10, also seems to participate in degradation of ABI5 protein (Smalle et al. 2003). A SUMO E3 ligase, SIZ1, has also been reported to be a negative regulator of ABI5 via the sumoylation of ABI5 (Miura et al. 2009). More recently, it was proposed that the interaction of the NINJA-related AFP proteins with ABI5 and co-repressor TPL-related proteins (TPRs) might be implicated in the regulation of ABI5-dependent gene expression (Pauwels et al. 2010). Thus, although various negative regulators associated with ABI5 protein degradation have been indicated to date, negative modulators of AREB/ABFs at the protein level have not been reported.

On the other hand, proteasome-dependent degradation has also been implicated in the positive regulation of ABA signaling. A RING finger E3 ligase, SDIR1, appears likely to act as a positive regulator upstream of both ABI5 and AREB/ABF family TFs (Zhang et al. 2007). However, the target of SDIR1 has not been identified. Thus, these findings support the notion that various bZIP family TFs (ABI5 and AREB/ABF) play key roles in ABRE-dependent gene expression associated with similar cellular dehydration in seeds and in the vegetative stage through different bZIP modulation mechanisms, in addition to the common core sensing system (PYR/PYL/RCAR-PP2C-SnRK2) (Fig. 2).

## Other TFs involved in ABA-mediated gene expression in response to osmotic stress during the vegetative stage of Arabidopsis

In addition to AREB/ABF bZIP TFs, other TFs have been reported to be involved in ABA-mediated gene expression. These TFs belong to several classes, including bZIP, AP2/ERF, MYB, NAC, HD-ZF, HD-Zip, bHLH, C2H2-ZF, B3, WRKY, and NF-Y (Table 1). Intriguingly, TFs associated with ABA-mediated transcription under osmotic stress conditions are relatively concentrated within particular clades in some TF families, including bZIP, NAC, and HD-Zip, whereas the associated TFs are comparatively scattered throughout the other families. Additionally, the expression of some TFs implicated in ABA-responsive gene expression does not appear to be significantly induced by exogenous ABA application. In this section, we will discuss the other TFs involved in ABA-mediated gene expression according to TF family.

### AP2/ERF TFs: complex involvement of AP2/ERFs in ABA-mediated gene expression

AP2/ERF proteins are TFs unique to plants and are encoded by more than 120 genes in Arabidopsis (Kim et al. 2006; Nakano et al. 2006). One class of AP2/ERF proteins that plays a central role in abiotic stress signaling consists of the DRE/CRT binding proteins, DREB1/CBFs. DREB1/CBF TFs can bind to the DRE/CRT motif (A/GCCGAC), a *cis*-acting element that functions in the expression of many stress-inducible genes in Arabidopsis (Agarwal et al. 2006; Kizis et al. 2001; Maruyama et al. 2004; Sakuma et al. 2002; Yamaguchi-Shinozaki and Shinozaki 2006). The genes, *DREB1A/CBF3*, *DREB1B/CBF1*, and *DREB1C/CBF2* are induced by cold (Liu et al. 1998), whereas *DREB1D/CBF4* is responsive to osmotic stress (Haake et al. 2002) and *DREB1E/DDF2* and *DREB1F/DDF1* are up-regulated by salinity stress (Magome et al. 2008). It has been reported that the dehydration-responsive *DREB1D/*

**Table 1** TFs implicated in ABA-mediated gene expression in response to osmotic stress during the vegetative growth stage in *Arabidopsis thaliana*

| Transcription factor | Accession no. | Classification | *cis*-element | Stress condition | Interactor |
|---|---|---|---|---|---|
| AREB1/ABF2 | At1g45249 | bZIP | ABRE | ABA, drought, salt | ARIA, CPK32, DREB1A, DREB2A, DREB2C |
| AREB2/ABF4 | At3g19290 | bZIP | ABRE | ABA, drought, salt | DREB1A, DREB2A, DREB2C |
| ABF3 | At4g34000 | bZIP | ABRE | ABA, drought, salt | DREB2C |
| CBF4/DREB1D | At5g51990 | AP2/ERF | DRE | ABA, drought | |
| DREB2A | At5g05410 | AP2/ERF | DRE | ABA, drought, heat, salt, $H_2O_2$ | AREB1/ABF2, AREB2/ABF4 |
| RAP2.6 | At1g43160 | AP2/ERF | GCC, CE1 | ABA, salt, JA, osmotic | |
| ABR1 | At5g64750 | AP2/ERF | | ABA, drought, salt, cold | |
| AtERF7 | At3g20310 | AP2/ERF | GCC | ABA, drought | PKS3/SnRK3.1/CIPK15, SIN3 |
| ADAP | At5g19330 | AP2/ERF | | ABA, drought, salt | ARIA |
| MYB2 | At2g47190 | R2R3-MYB | MYBR | ABA, drought | |
| MYB96 | At5g62470 | R2R3-MYB | | ABA, drought | |
| MYB102 | At4g21440 | R2R3-MYB | ABRE, CE1, W-box | ABA, salt, JA, wounding | |
| BOS1/MYB108 | At3g06490 | R2R3-MYB | | ABA, drought, salt, JA, pathogen, $H_2O_2$ | |
| MYB44 | At5g67300 | R2R3 MYB | | ABA, drought, salt, JA | |
| MYB41 | At4g28110 | R2R3-MYB | | ABA, drought, salt | |
| ANAC019 | At1g52890 | NAC | CATGTG | ABA, drought, salt | RHA2a |
| NAC3/ANAC055 | At3g15500 | NAC | CATGTG | ABA, drought, salt | RHA2a |
| RD26/ANAC072 | At4g27410 | NAC | CATGTG | ABA, drought, salt | |
| ATAF1/ ANAC002 | At1g01720 | NAC | | ABA, drought, salt | |
| AtNAC2/ ANAC092/ ORE | At3g15510 | NAC | | ABA, salt, ET, auxin | |
| ZFHD1 | At1g69600 | HD-ZF | ZFHDRS | ABA, drought, salt | |
| ATHB5 | At5g65310 | HDZip | | ABA | |
| ATHB6 | At2g22430 | HDZip | CAATTATTA | ABA, drought | ABI1 |
| ATHB7 | At2g46680 | HDZip | | ABA, drought | |
| ATHB12 | At3g61890 | HDZip | | ABA, drought, GA | |
| MYC2 | At1g32640 | bHLH | G-box like MYCR | ABA, drought, salt, pathogen, wounding, JA, ET, SA | JAZ |
| AIB | At2g46510 | bHLH | | ABA, drought | |
| AZF2 | At3g19580 | C2H2 ZF | | ABA, drought, salt | |
| SAZ | At4g17810 | C2H2 ZF | | ABA, drought, salt, ozone, UV-B, SA | |
| ABO3/WRKY63 | At1g66600 | WRKY | W-box | ABA, drought | |
| NFYA5 | At1g54160 | NF-Y | CCAAT | ABA, drought | |

See main text for details and references

*CBF4* and the cold-responsive *DREB1A-C/CBF1-3* are also induced by exogenous ABA (Haake et al. 2002; Knight et al. 2004). Although the dehydration-induced gene expression of *DREB1D/CBF4* is evidently mediated by ABA, it remains controversial whether and how the expression of *DREB1A-C/CBF1-3* genes is involved in ABA signaling in response to cold stress (Knight et al. 2004; Yamaguchi-Shinozaki and Shinozaki 2006). The other classes of *DREB* family genes, *DREB2A* and *DREB2B*, are strongly induced by osmotic and high-temperature stresses, while *DREB2A* is slightly up-regulated by ABA (Liu et al. 1998; Nakashima et al. 2000; Sakuma et al. 2006). A detailed analysis of an *RD29A* promoter that is responsive to multiple abiotic stressors has indicated that DRE/CRT can function as a CE in promoters of ABA-regulated genes (Nakashima et al. 2006; Narusaka et al. 2003). Recently, DREB1A/CBF3, DREB2A, and DREB2C proteins have been reported to physically interact with

AREB/ABF proteins (Lee et al. 2010c). Together, these observations support the view that DREB/CBFs and AREB/ABFs may interact to control ABA-regulated gene expression.

During cold acclimation, two AP2/ERF TFs, RAP2.1 and RAP2.6, have been identified as subregulons of the DREB/CBF activators (Fowler and Thomashow 2002). Recent data also show that RAP2.1 represents a negative transcriptional regulator in response to cold and drought stresses in an ABA-independent manner (Dong and Liu 2010). On the other hand, RAP2.6, which can interact with either GCC (AGCCGCC) or CE1 (TGCCACCGG) *cis*-elements, acts as a positive regulator of osmotic stress responses in an ABA-dependent manner (Zhu et al. 2010). In addition, RAP2.6 participates in COI1-dependent JA-responsive transcription (Wang et al. 2008) and in defense response to bacterial pathogens (He et al. 2004). These findings suggest a more complex involvement of ABA-responsive gene expression in the DREB/CBF-mediated transcriptional network.

The AP2/ERF TFs belonging to the other class, ABR1 (Pandey et al. 2005) and AtERF7 (Song et al. 2005), have been reported to be negative regulators of ABA-responsive gene expression. ABR1 is responsive to ABA and to stress conditions including cold, high salinity, and drought, while AtERF7 is not induced by ABA or by other abiotic stressors. AtERF7 appears to bind to a GCC-box (GCCGCC) motif (Guo et al. 2002), which is known to be involved in various signaling processes (Rushton et al. 2002). Moreover, AtERF7 is regulated through phosphorylation by the protein kinase PKS3/SnRK3.1/CIPK15, which physically interacts with ABI2 and is likely a negative regulator of ABA responses (Guo et al. 2002). Ectopic expression of *AtERF7* in transgenic Arabidopsis plants reduces ABA responses in guard cells and decreases drought tolerance, whereas reductions in *AtERF7* expression cause ABA hypersensitivity in guard cells, seed germination, and seedling growth. On the other hand, an AP2/ERF TF, ADAP (ARIA-interacting Double AP2 domain Protein), is considered to be a positive regulator of ABA responses (Lee et al. 2009), although there is no direct evidence showing the involvement of ADAP in ABA-responsive gene expression. ADAP interacts with ARIA (Arm Repeat Protein Interacting with ABF2), which interacts with AREB1/ABF2 and NPK6 (Kim et al. 2004a; Lee et al. 2009, 2010b). Although the level of *ADAP* mRNA is not changed significantly by ABA or by abiotic stressors, *adap* knockout mutant plants show partial insensitivity to ABA and decreased drought tolerance.

Several AP2/ERF TFs have been reported to be mediators of ABA responses, mainly during germination and the early seedling stage. ABI4 (Finkelstein et al. 1998) and WRI1 (Cernac et al. 2006; Cernac and Benning 2004) function as positive and negative regulators of ABA and sugar responses, respectively. A recent report has demonstrated that the CE1-like CACCG motif in the ABI5 promoter is required for ABI4 transactivation in Arabidopsis protoplasts (Bossi et al. 2009). CHO1 (Nambara et al. 2002) is probably involved in the ABA-dependent repression of GA biosynthesis (Yano et al. 2009) as well as sugar and nitrate responses in an ABI4-dependent manner (Yamagishi et al. 2009).

## MYB TFs: diverse R2R3-MYBs participate in the crosstalk between ABA signaling and other stress signaling pathways

*MYB* genes were initially identified as oncogenes originating from a retrovirus in mammalian cells (Klempnauer et al. 1982). In plants, unlike in animals (R1R2R3-MYB), most MYB proteins belong to the R2R3-type MYB family. These proteins share conserved MYB DNA-binding domains consisting of two 50- to 53-amino acid imperfect repeats that form the helix-turn-helix motifs R2 and R3 (Kranz et al. 1998; Stracke et al. 2001). The Arabidopsis genome encodes 126 *R2R3-MYB* genes (Dubos et al. 2010). Several *R2R3-MYB* genes have been reported as important mediators of ABA-mediated gene expression under environmental stress conditions in Arabidopsis. MYB2 is responsive to dehydration and ABA in cooperation with a basic helix-loop-helix (bHLH) TF (MYC2) and activates the dehydration- and ABA-inducible expression of the *RD22* gene (Abe et al. 1997, 2003). Transgenic Arabidopsis plants that overexpress both *MYB2* and *MYC2* exhibit greater sensitivity to ABA and enhanced osmotic stress tolerance compared to wild-type plants. MYB96 also appears to mediate ABA signaling via the expression of *RD22* in regulating drought stress response (Seo et al. 2009). *MYB96* is responsive to ABA and drought stress and is induced by IAA, primarily in roots. The *MYB96*-overexpressing Arabidopsis mutant displays enhanced drought tolerance with reduced lateral roots, whereas MYB96 deficiency results in increased sensitivity to water deficit. The MYB96-mediated ABA signaling implicated in control of the *RD22* gene are transduced via an auxin signaling pathway that involves a subset of *GH3* genes encoding auxin-conjugating enzymes. These observations may support the view that MYB96 functions as a molecular convergence point between the ABA signaling and auxin signaling pathways.

Other R2R3 MYB TFs participate in the crosstalk involved in ABA-mediated gene expression. *MYB102* has been shown to respond to ABA, JA, salt stress and wounding (Denekamp and Smeekens 2003). The combined studies suggest that MYB102 functions in the response of plants to dehydration after wounding via conserved promoter elements, such as ABRE-CE1 and W-box, and their interacting factors (Denekamp and Smeekens 2003). However, MYB102 is also associated with defensive responses against insect herbivores (De Vos et al. 2006). One of the closet homologs of MYB102, MYB41, appears to play a distinct role in stress responses. The *MYB41* transcript is induced to a high level by drought, salt and ABA treatment (Cominelli et al. 2008). Analysis of transgenic lines that over-express *MYB41* has revealed that MYB41 regulates wax accumulation and cell expansion, implying that MYB41 may play a role in cell wall modification, cuticle synthesis and cuticle deposition in response to abiotic stress. On the other hand, BOS1/MYB108 is induced by necrotrophic pathogens through a JA-mediated signaling pathway (Mengiste et al. 2003). BOS1/MYB108 appears to function as a positive transcriptional regulator of JA- and ABA-inducible target genes, whose expression plays an important role in abiotic and biotic stress tolerance. Disruption of BOS1/MYB108 results in increased sensitivity to necrotrophic pathogens and in impaired drought, salinity, and oxidative stress tolerance. In addition, MYB44 is probably also involved in a convergence point of stress signaling pathways. Several transcriptome analyses have indicated that *MYB44* mRNA accumulation is induced in most tissues by a variety of hormone treatments (ABA, IAA, ET, JA and GA), environmental conditions (drought, high salinity, and low temperature), and pathogen infections (Jung et al. 2007, 2008; Kranz et al. 1998; Yanhui et al. 2006). The over-expression of *MYB44* in transgenic Arabidopsis plants enhances sensitivity to ABA and ABA-induced stomatal closure and tolerance to drought and salt stresses compared to wild-type plants (Jung et al. 2008). Furthermore, this over-expression suppresses JA-responsive gene activation (Jung et al. 2010), supporting the view that MYB44 is involved in mutual antagonistic actions between the ABA- and JA-mediated signaling pathways. Together, these observations suggest that diverse R2R3 MYB TFs might function as key mediators of stress responses through complex activities spanning multiple stress signaling pathways (for reviews: Fujita et al. 2006, 2009a, b).

## NAC TFs: ABA-responsive NACs mediate versatile stress responses via multiple hormonal signaling

NAC-family proteins contain N-terminal NAC DNA-binding domains. This plant-specific gene family consists of more than 100 members with diverse cellular functions (Olsen et al. 2005; Ooka et al. 2003). *ANAC019*, *ANAC055*, *RD26/ANAC072*, and *ATAF1* are all up-regulated by ABA, high salinity, and dehydration (Fujita et al. 2004; Tran et al. 2004) and are found in the same clade in phylogenetic analyses (Fujita et al. 2004). It is likely that ANAC019, ANAC055, and RD26/ANAC072 proteins all bind to the CATGTG motif and regulate several stress-inducible genes associated with drought tolerance (Tran et al. 2004). In addition, these NAC TFs and ZFHD1 are induced by ABA and osmotic stress and appear to cooperatively activate the expression of stress-responsive genes (Tran et al. 2007). Meanwhile, RD26/ANAC072 and ANAC019 are positive regulators of ABA signaling under stress conditions (Fujita et al. 2004; Jensen et al. 2010). Ectopic expression of *RD26/ANAC072* or *ANAC019* in transgenic plants enhances sensitivity to ABA, while *RD26/ANAC072*-repressed plants are insensitive. Large-scale transcriptome analyses of both types of plants have revealed that RD26/ANAC072-regulated genes are involved in the detoxification of ROS, defense and senescence in an ABA-dependent manner (Fujita et al. 2004). In contrast, ATAF1 can be regarded as a negative regulator of ABA signaling and is required for the maintenance of effective penetration resistance during non-host biotrophic pathogen attack (Jensen et al. 2008). On the other hand, both ANAC019 and ANC055, which can interact with RHA2a, which is a C2H2-type RING finger protein, are active in E3 ligase modulation of ABA signaling, playing dual roles in the regulation of ABA and JA responses (Bu et al. 2008, 2009; Greve et al. 2003). Moreover, it has been shown that *AtNAC2/ANAC092/ORE1*, which is induced by salt stress and by the plant hormones ABA and NAA as well as the ethylene precursor ACC, seems to function downstream of the ethylene and auxin signaling pathways in lateral root development and salinity responses (He et al. 2005). A recent report further suggests that the expression of *AtNAC2/ANAC092/ORE1* is induced during leaf aging by EIN2 but is negatively regulated by *miR164* (Kim et al. 2009). As described here, ABA-responsive NAC family TFs are likely to function in versatile stress responses via multiple hormonal signaling pathways.

## HD-Zip TFs: Class I HD-Zip TFs engage in ABA-mediated stress responses

Plant-specific HD-Zip proteins, which are characterized by their homeodomain and leucine zipper motif, are encoded by 47 genes in Arabidopsis (Elhiti and Stasolla 2009). Based on sequence analyses, HD-Zip TFs are classified into four different groups, HD-Zip I–IV. HD-Zip Class I contains seventeen members in Arabidopsis and is generally involved in abiotic stress responses. *ATHB6* (Söderman et al. 1999), *ATHB7* (Söderman et al. 1996), and *ATHB12* (Henriksson et al. 2005; Lee and Chun 1998) are highly induced by exogenous ABA and osmotic stress. ATHB5, another member of this class, functions as a positive regulator of ABA responsiveness during seedling establishment (Johannesson et al. 2003). ATHB6 binds to an AT-rich *cis*-element (CAATTATTA) and interacts with ABI1 (Himmelbach et al. 2002). ATHB6 can also heterodimerize with ATHB5 and possibly with other HD-Zip TFs (Johannesson et al. 2003). Constitutive expression of ATHB6 in transgenic Arabidopsis plants decreases their sensitivity to ABA during germination and stomatal closure in a subset of ABI1-dependent responses, suggesting that ATHB6 acts as a negative regulator downstream of ABI1 in the ABA signaling pathway (Himmelbach et al. 2002). Recently, it has been reported that ATHB12 may represent a negative regulator of *GA 20-oxidase* gene expression in inflorescence stems (Son et al. 2010). Thus, these findings demonstrate that several Class I HD-Zip TFs mediate ABA-responsive transcription associated with cellular dehydration.

### bHLH TFs: MYC2 is a key regulator in multiple stress and light signaling pathways

Proteins of the bHLH family are present in all eukaryotes and are encoded by 147 genes in Arabidopsis (Toledo-Ortiz et al. 2003). A canonical bHLH domain consists of an N-terminal stretch of about 18 hydrophilic and basic amino acids followed by two regions of hydrophobic residues predicted to form amphipathic $\alpha$-helices separated by an intervening loop. Two bHLH TFs, MYC2 and AtAIB, have been reported to be implicated in ABA-mediated gene expression in Arabidopsis. MYC2 is synonymous with RD22BP1 (Abe et al. 1997), RAP-1 (de Pater et al. 1997), AtbHLH006 (Heim et al. 2003), and ZBF1 (Yadav et al. 2005). Genetic analysis of *jin1/jai1* mutants has shown that *JIN1/JAI1* encodes *MYC2* (Lorenzo et al. 2004). DNA binding assays have demonstrated that MYC2 preferentially interacts with the G-box-like MYC recognition (MYCR) sequences 5′-CACATG-3′ (Abe et al. 1997), 5′-CACNTG-3′ (de Pater et al. 1997), 5′-(T/C)ACGTG-3′ (Yadav et al. 2005), and 5′-CACHTG-3′ (Dombrecht et al. 2007), apparently indicating that the MYC2 core binding sequence is 5′-CACNTG-3′. MYC2 was initially characterized as a positive regulator of ABA-inducible genes, including *RD22* and *ADH1*, under osmotic stress conditions (Abe et al. 1997; 2003). Since then, a large body of evidence has revealed that MYC2 is the key regulator of crosstalk in abiotic and biotic stress responses and in light signaling pathways via hormone signaling pathways, including ABA, JA, ethylene, and SA (Fujita et al. 2009a, b). Another bHLH TF, AtAIB, likely acts as a transcriptional activator of ABA-mediated responses to drought stress (Li et al. 2007). Although only a few members of the bHLH family are known to be involved in ABA-mediated gene expression, these data collectively suggest that MYC2 plays an important role in the convergence point between multiple stress and light signaling pathways.

### C2H2 ZF TFs: mediators in crosstalk among the ABA, JA and SA signaling pathways

C2H2 zinc finger proteins are involved in RNA metabolism, chromatin remodeling and transcriptional regulation (Englbrecht et al. 2004). The Arabidopsis genome encodes 176 C2H2-type zinc finger proteins, many of which appear to function as TFs. Two C2H2 zinc finger proteins that contain an ERF-associated amphiphilic repression domain, AZF2 and SAZ, have been reported to be negative regulators implicated in ABA-mediated gene expression under osmotic stress conditions. AZF2 was originally identified as an ABA-responsive transcriptional repressor under stress conditions (Sakamoto et al. 2004). Dehydration-responsive *AZF2* expression is partially mediated by ABI1, whereas *AZF2* expression is induced by high salinity in an ABA-independent manner. In addition, AZF2 seems to function as a negative regulator of ABA signaling during seed germination (Drechsel et al. 2010) and is associated with JA biosynthesis and signaling (Pauwels and Goossens 2008; Pauwels et al. 2008).

Another C2H2 zinc finger protein, SAZ, appears to be a negative regulator of some ABA-mediated stress-responsive gene expression (Jiang et al. 2008). *SAZ* expression is down-regulated in response to abiotic stresses, including salinity, drought, ozone, and UV-B irradiation, and in response to plant hormones, including ABA and SA. It is likely that the SAZ TF functions downstream of the AREB/ABFs as a negative regulator of some AREB/ABF-regulated genes, such as *RD29B* and *RAB18*. SA-responsive down-regulation of *SAZ* is mediated by NPR1 and is a key regulator in the SA signaling pathway and systemic acquired resistance, implying that SAZ may function in the crosstalk between the ABA and SA signaling pathways.

### AFL B3 domain TFs: key regulators in seed development and maturation

The B3 domain was initially characterized in the VP1 (VIVIPAROUS1) protein from maize, which contains three basic regions called B1, B2, and B3 (Suzuki et al. 1997). Although five major classes of B3 domain TFs have been reported to date, one class of B3 domain TFs belonging to the ABI3 family, which recognizes the Sph/RY element of the CATGCA motif, is known to comprise important mediators involved in ABA signaling (Romanel et al. 2009). These ABI3 family-B3 domain TFs (AFL B3 TFs), ABI3, FUS3, and LEC2, have been shown to function as key regulators of hormone signaling pathways involved in seed embryo maturation, including ABA, GA, and auxin (Suzuki and McCarty 2008). Recently, these three B3 domain TFs have been identified as target genes of AGL15, a MADS domain TF that promotes somatic embryogenesis by binding DNA and regulating gene expression (Zheng et al. 2009). Although these AFL B3 TFs have no been reported to be major players in the response to osmotic stress during the vegetative phase, it appears that AFL B3 TFs are significant for the difference in ABA-mediated transcription between the vegetative phase and seeds.

## WRKY and NF-Y TFs: recently discovered TF families involved in ABA signaling in response to osmotic stress in Arabidopsis

Plant-specific WRKY TFs, which exhibit high affinity and sequence specificity to the *cis*-element W-box (TTGACC/T), make up a large gene family of 74 members in Arabidopsis (Rushton et al. 2010). The defining characteristic of WRKY TFs is their DNA-binding domain, which is composed of the N-terminal conserved amino acid sequence WRKYGQK and a zinc-finger-like motif. Although the majority of reports regarding WRKY TFs to date have revealed prominent roles in transcriptional reprogramming associated with plant immune responses, little is known about their role in ABA-mediated gene expression and abiotic stress responses in Arabidopsis. However, Ren et al. (2010) have recently reported that ABO3/WRKY63 may act as a mediator in response to ABA and drought stress. *AREB1/ABF2* expression is partially impaired in *abo3* mutants during early ABA treatment. In addition, ABO3 is capable of binding to the W-box with a TGAC core sequence in the AREB1/ABF2 promoter in vitro. Moreover, *abo3* mutants display enhanced sensitivity to ABA, whereas *ABO3*-overexpressing transgenic plants show no phenotypic change.

NF-Y TFs, which favorably bind to a *cis*-element CCAAT box, are found in all eukaryotes (Siefers et al. 2009). NF-Y TFs are complexes of three different subunits, designated NF-YA, NF-YB, and NF-YC. The Arabidopsis genome encodes 10 NF-YAs, 13 NF-YBs, and 13 NF-YCs (Gusmaroli et al. 2002). Among them, *NFYA5* is strongly induced by ABA treatment and drought stress in an ABA-dependent manner (Li et al. 2008). *NFYA5* expression has been observed in guard cells and vascular tissues and is likely modulated by *miR169a*, which is down-regulated by drought stress. Analysis of *miR169a* or *NFYA5* overexpressing lines and *nfya5* knockout plants indicates that NFYA5 plays an important role in controlling stomatal aperture and drought tolerance.

## Perspectives

ABA-mediated transcriptional regulation plays a crucial role in many cellular processes in plants. Recently, the core ABA signaling complex has been described in detail (for reviews: Cutler et al. 2010; Hubbard et al. 2010; Raghavendra et al. 2010; Weiner et al. 2010). In addition, several regulatory factors, including TFs, and crosstalk between the pathways that link various hormonal and stress signaling pathways implicated in ABA-dependent gene expression have been identified and characterized. Among the regulatory factors studied to date, TFs involved in ABA-mediated gene expression are increasingly recognized as promising candidates for the creation of useful transgenic crops that can tolerate osmotic stress through appropriate temporal and spatial regulation of the expression of target genes. A combination of artificial promoter techniques capable of regulating spatial and temporal expression will allow us to create ideal crops with versatile tolerance to environmental stresses. Recently, it has been reported that ABA is generated and functions in the stress signaling pathways of mammals (Bassaganya-Riera et al. 2010; Bruzzone et al. 2007). We believe that studies of ABA-mediated gene expression in plants will contribute to new directions in medical science as well as to efficient, economical and environmentally sound crop production.

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